JOURNAL OF AGRICULTURAL RESEARCH

VOL. 65 WASHINGTON, D. C., DECEMBER 1, 1942 No. 11

INHERITANCE AND INTERRELATIONSHIP OF COMPONENTS OF QUALITY, COLD RESISTANCE, AND MORPHOLOGICAL CHARACTERS IN WHEAT HYBRIDS[1]

By W. W. WORZELLA

Associate in agronomy, Purdue University Agricultural Experiment Station

INTRODUCTION

In the soft red winter wheat region one of the main problems in the breeding program is that of developing new varieties that not only possess suitable quality but also are winter hardy. In order to "build up" or synthesize such new varieties most directly it is essential to obtain information on the mode of inheritance of quality and winter hardiness, and to determine the extent of correlation or linkage, if any, among these characters.

This paper reports a study of the inheritance and interrelationship of components of quality, cold resistance, and morphological characters in wheat (*Triticum aestivum* L.) hydrids. It was carried out on 600 hybrids and the parent stocks originating from a triangular cross of American Banner, Trumbull, and Michikof, grown at Lafayette, Ind., in 1937–38 and repeated in 1939–40.

REVIEW OF LITERATURE

Several reports have been published on the inheritance of components of quality, cold resistance, and morphological characters in wheat, but few data are available on the interrelationships of these characters, especially with hybrid populations selected at random. Since 11 different characters are taken up in this report, only the more pertinent papers will be reviewed.

Workers have used loaf volume, fermentation time, extensimeter, and swelling number as indices of gluten strength. Meneret (*32*)[2] and Rosenstiel (*36*) found two pairs of factors, while Worzella (*43*) found three pairs of factors to govern the mode of inheritance of this character. Data obtained by Alabouvette (*5*), Ausemus et al. (*8*), Cutler and Worzella (*20*), Hayes et al. (*27*), Saunders (*37, 38*), and Worzella and Cutler (*46*) indicate that the inheritance of gluten strength is governed by multiple factors.

Aamodt and Torrie (*2*), Biffen (*9, 10*), Bryan and Pressley (*12, 13*), and Howard and Howard (*28*) reported that a single genetic factor conditioned the inheritance of kernel texture. Two factors for kernel texture were reported by Aamodt and Torrie (*2*), and Freeman (*22*), while polymeric factors were found by Aamodt et al. (*3*), Clark et al. (*16*), Harrington (*24*), Hayes (*25*), and Hayes et al. (*27*).

[1] Received for publication Jan. 24, 1942. Journal Paper No. 2 of the Purdue University Agricultural Experiment Station.

[2] Italic number in parentheses refer to Literature Cited, p. 520.

494656—42——1

Working with a series of varieties and hybrid strains, Whiteside (*41*), Whiteside et al. (*42*), and Worzella and Cutler (*45*, *46*) showed that the carotenoid pigment content in wheat is genetic in nature. Clark and Smith (*19*) and Markley (*31*) reported that polymeric factors govern the inheritance of carotenoid content in durum wheats.

Multiple factor inheritance for crude protein content was indicated by Aamodt and Torrie (*2*), Ausemus et al. (*8*), Clark (*15*), Clark et al. (*16*), Clark and Hooker (*17*), Clark and Quisenberry (*18*), Clark and Smith (*19*), Hayes et al. (*27*), and Zinn (*49*).

The mode of inheritance of kernel weight was reported by Jasnowski (*29*, *30*) to be governed by three factor pairs.

Åkerman (*4*), Hayes and Aamodt (*26*), Nilsson-Ehle (*33*), Quisenberry (*34*), Quisenberry and Clark (*35*), and Worzella (*44*), working with different wheat crosses, concluded that cold resistance is a heritable character and is controlled by many genetic factors.

Aamodt and Torrie (*2*), Ausemus et al. (*8*), Clark (*15*), Clark and Smith (*19*), Goulden et al. (*23*), Hayes et al. (*27*), Waldron and Mangels (*40*), Whiteside et al. (*42*), and Zinn (*49*) studied the interrelationship of factors for quality, such as protein content, loaf volume, texture, color, test weight, etc., on a series of varieties and selected hybrid strains. Data obtained by these workers show a general tendency for an association between protein content and loaf volume, carotenoid content and crumb score, and kernel texture and protein content; however, many of the correlation coefficients were low in magnitude while others were not significant. Intercharacter correlations between other components of quality usually were not significant or consistent under different conditions.

Many investigators have reported that the mode of inheritance of glume and kernel color is governed by one, two, or three pairs of genetic factors. Ausemus (*7*) and Churchward (*14*) showed that one factor pair governs the inheritance of coleoptile color, while Quisenberry (*34*) found two factor pairs. The inheritance of straw color was reported by Torrie (*39*) to be monogenic. Churchward (*14*) found a strong correlation between straw and coleoptile color.

MATERIALS AND METHODS

The experiments here reported were conducted with F_3, F_4, and parent rows grown under field conditions during 1937–38 and repeated in 1939–40. The three common wheat varieties American Banner, Trumbull, and Michikof were used as parents in this study. Table 1 shows the contrasting characters of the three varieties.

Reciprocal crosses between pure lines of American Banner, Trumbull, and Michikof were made in the greenhouse in 1935 and F_1 plants were grown during 1935–36. The F_2 generation was grown in the field at the soils and crops farm at Lafayette, Ind., during 1936–37. The kernels were spaced at 4-inch intervals in 18-foot rows 1 foot apart.

TABLE 1.—*Contrasting characters of the parent varieties American Banner, Trumbull, and Michikof*

Contrasting characters	Variety			Difference necessary for significance between 2 means (Odds 19:1)
	American Banner	Trumbull	Michikof	
Gluten strength ..... minutes	18.8	31.4	195.6	[1] 6.42
Particle-size index ..... percent	16.5	17.8	8.8	.87
Carotenoid content ..... p. p. m.	1.87	1.95	2.09	.08
Protein content ..... percent	9.1	9.6	10.6	.30
1,000-kernel weight ..... grams	36.0	35.2	31.9	1.00
Test weight ..... pounds	57.5	59.2	60.2	.67
Cold resistance ..... percent	20.6	24.2	50.9	1.09
Glume color	Brown	White	White	
Kernel color	White	Red	Red	
Coleoptile color	Green	Purple	Green	
Straw color	White	Purple	White	

[1] Percentage below the mean (Worzella, *43, pp. 707–708*).

A random selection of the F_2 populations was made as a basis for continuing the study in the F_3 generation. Two hundred F_2 plants were selected from each of the three crosses. In the fall of 1937, 50 grains from each F_2 plant were used to seed an F_3 progeny row by spacing the kernels at 3-inch intervals in 12-foot rows 1 foot apart. Check rows of the parent varieties, sown in the same manner, were alternated every tenth row. Following a favorable growing season, from 35 to 48 plants per row were available for harvest in 1938. Representative wheat samples for each row were obtained by harvesting 3 heads from each plant and threshing them in bulk. In addition, 1 head from each F_3 hybrid plant was selected for F_4 progeny tests. The 666 composite samples, representing the parents and F_3 progenies of the 1938 crop, were analyzed for several components of quality and cold resistance.

In the fall of 1939, 200 grains from each F_3 family were planted in 6-foot rows 1 foot apart. Check rows of the parent varieties, sown in the same manner, were alternated every tenth row. In addition, several F_3 families that showed wide differences in respect to quality were further progeny tested in 4-foot head rows in the F_4 generation. Each row was harvested separately and threshed in bulk. Representative samples from each row were used to conduct the quality studies of the 1940 crop.

Gluten strength was determined by the wheat-meal fermentation-time test as described by Cutler and Worzella (*21*). Granulation or the particle-size index of wheat meal was determined according to the method developed by Worzella and Cutler (*47*). The method reported by Binnington and Geddes (*11*) with some minor modifications was used for determining the carotenoid content of wheat. A 10-gm. sample of finely ground wheat meal was placed in a 4-ounce bottle containing 50 cc. of water-saturated butanol and allowed to stand for 16 hours with occasional shaking. Clarification was effected by filtration through No. 1 Whatman paper. The yellow pigment content of the extract was determined in a 2-cm. cell, using a KWSZ photometer. Pure beta-carotene was used to prepare a series of known standards. By means of a conversion table the percentage transmittancy readings were expressed as parts of carotene per million parts of wheat meal. Crude-protein determinations were made

according to the method outlined in Cereal Laboratory Methods (*6*). Protein data are reported on a 13.5 percent moisture basis. The relative test weights of the small samples of wheat were determined by the method developed by Aamodt and Torrie (*1*). Cold-resistance studies were conducted under artificial freezing tests according to the method described by Worzella and Cutler (*48*).

EXPERIMENTAL RESULTS

Genetic analyses and experimental data on gluten strength, granulation, carotenoid pigment content, crude protein, kernel weight, test weight, cold resistance, and several morphological characters are presented in the order named. Following these, the relationships between the characters are reported.

INHERITANCE OF CHARACTERS

Gluten Strength

To study the inheritance of gluten strength, fermentation-time tests were made on the grain obtained from F_3 and F_4 hybrids and parents, originating from a triangular cross of American Banner, Trumbull, and Michikof, grown at Lafayette, Ind., in 1937–38 and

TABLE 2.—*Frequency distribution of fermentation time for gluten strength of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses and of the parent varieties*

Parent or cross	Generation	Year	Lines in class center for fermentation time in number of minutes stated [1]									Lines	Mean	Coefficient of variability [2]
			18.0	24.4	33.1	44.9	60.8	82.4	111.8	151.4	205.2			
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Min.*	
American Banner	P_1	1938	20	2								22	18.5	8.4
Do	P_1	1940	18	4								22	19.1	8.6
Trumbull	P_1	1938		1	21							22	32.7	8.9
Do	P_1	1940		7	15							22	30.1	13.9
Michikof	P_1	1938								3	19	22	196.9	10.5
Do	P_1	1940								4	18	22	194.2	11.3
AB × T	F_3	1938	54	120	25	1						200	23.5	17.4
Do	F_4	1940	51	111	37	1						200	24.0	18.6
AB × M	F_3	1938	1	2	37	75	54	14	9	6	2	200	52.2	32.8
Do	F_4	1940		3	31	77	46	17	15	10	1	200	54.1	33.0
T × M	F_3	1938			5	36	64	42	29	21	3	200	74.0	33.8
Do	F_4	1940			11	27	40	46	35	33	8	200	82.2	37.7
Pedigree No.:														
76	F_4	1940	24	2								26	17.2	8.2
635	F_4	1940	7	13	1							21	22.4	15.7
299	F_4	1940			1	4	14	5	3	1		28	66.2	27.9
650	F_4	1940				1	3	3	6	4	1	18	101.0	33.3
603	F_4	1940							1	11	4	16	166.5	13.6
266	F_4	1940								1	21	22	202.3	8.8

[1] A logarithm of 0.132 was used in calculating class intervals (Worzella, *43*, *p. 707*).
[2] Percentage below the mean (Worzella, *43*, *pp. 707–708*).

1939–40. The data obtained were arranged in frequency distributions and are shown in table 2 and in figure 1.

The results show that the three parent varieties are widely different in gluten strength. The average time of disintegration for dough balls made from American Banner, Trumbull, and Michikof, was about 19, 31, and 195 minutes, respectively.

The gluten quality of the F_3 families, in each cross, varied all the way from that of the weaker to that of the stronger parent. The frequency distributions of fermentation time for the hybrids from the

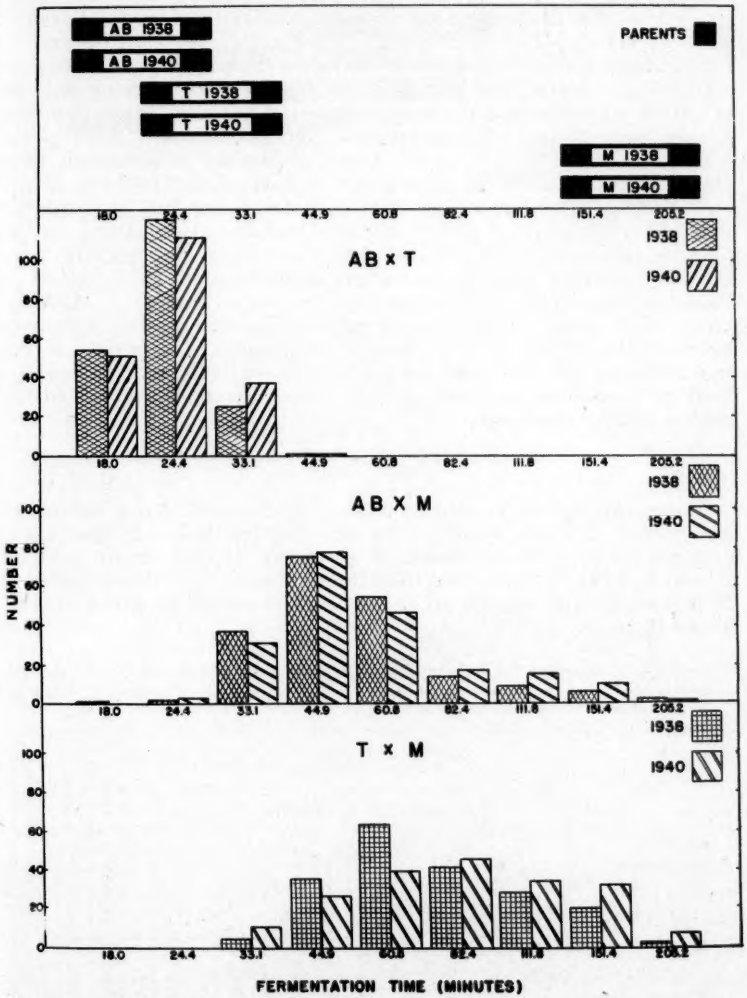

FIGURE 1.—Range in fermentation time for gluten strength of the parent varieties American Banner, Trumbull, and Michikof, and F_2 distribution of fermentation time for gluten strength of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses. Crops of 1938 and 1940.

American Banner × Michikof and Trumbull × Michikof crosses, approach normal curves. There is, however, a preponderance of hybrids toward the short time side of the frequency distribution

which may be due to the effect of environmental conditions favoring the development of weak gluten wheats. F_4 families just as weak as American Banner and just as strong as Michikof in gluten strength were recovered. Some of the F_4 lines (76 and 266) bred comparatively true, the range for time being no greater than for the parents, while others varied as much as the F_3 generation (F_2 distribution).

The sharp rise in the variability between the parents and later generations, the recombination of families possessiong various degrees of gluten strength, and the reappearance of parental types, indicate clearly the existence of segregation of genetic factors. Since about one-fourth of the families in the American Banner × Trumbull cross possess approximately the same gluten quality as that found in American Banner, it would appear that 1 major factor pair governs the mode of inheritance of gluten strength in this cross. Based on the number of parental recombinations, when only 200 families were studied, the data indicate that about 4 factor pairs are involved in the inheritance of gluten strength in the American Banner × Michikof cross, while about 3 factor pairs may explain the genetic difference between the Trumbull and Michikof varieties. The writer is not unmindful of the fact that such a genetic analysis may not always hold true, since environment greatly influences the expression of the gluten quality character.

Granulation

Meal granulation, or degree of particle fineness, is an important component of wheat quality. In studying its mode of inheritance, granulation tests were conducted on finely ground wheat meal of F_3 and F_4 hybrids originating from three crosses. The data, expressed in percentage and designated as particle-size index, are given in table 3 and figure 2.

Table 3.—*Frequency distribution of particle-size index of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses and of the parent varieties*

Parent or cross	Generation	Year	Lines in class center for particle-size index of wheat meal in percentage stated											Lines	Mean	Standard deviation
			23.4	21.8	20.2	18.6	17.0	15.4	13.8	12.2	10.6	9.0	7.4			
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	
American Banner	P_1	1938	----	----	----	----	4	10	7	1	----	----	----	22	15.1	1.35
Do	P_1	1940	----	----	5	6	8	3	----	----	----	----	----	22	17.9	1.62
Trumbull	P_1	1938	----	----	----	7	9	5	1	----	----	----	----	22	17.0	1.24
Do	P_1	1940	1	2	2	9	8	----	----	----	----	----	----	22	18.7	1.88
Michikof	P_1	1938	----	----	----	----	----	----	----	----	1	9	12	22	8.3	.81
Do	P_1	1940	----	----	----	----	----	----	----	2	4	14	2	22	9.4	1.23
AB × T	F_3	1938	----	----	9	52	88	45	3	3	----	----	----	200	17.1	1.48
Do	F_4	1940	13	39	68	62	16	2	----	----	----	----	----	200	19.9	1.71
AB × M	F_3	1938	----	----	2	6	18	19	29	43	32	31	20	200	12.2	3.10
Do	F_4	1940	----	----	----	4	15	18	36	47	42	30	8	200	12.3	2.64
T × M	F_3	1938	----	----	1	5	19	22	29	43	34	31	16	200	12.3	2.98
Do	F_4	1940	----	----	4	10	15	34	47	35	31	20	4	200	13.3	2.84
Pedigree No.:																
299	F_4	1940	----	1	1	4	10	9	2	1	----	----	----	28	16.6	1.98
216	F_4	1940	----	----	----	3	16	10	----	----	----	----	----	29	16.6	1.02
339	F_4	1940	----	----	----	2	3	4	6	4	3	3	1	26	13.4	3.05
635	F_4	1940	----	----	----	----	----	1	2	3	8	5	2	21	10.7	2.05
322	F_4	1940	----	----	----	----	----	----	----	----	3	5	22	30	8.0	1.07

A comparison of the results of the two seasons shows that the wheat of the 1940 crop produced a finer meal or flour than that harvested in 1938. The average particle-size index of 16.5 and 17.8 for American

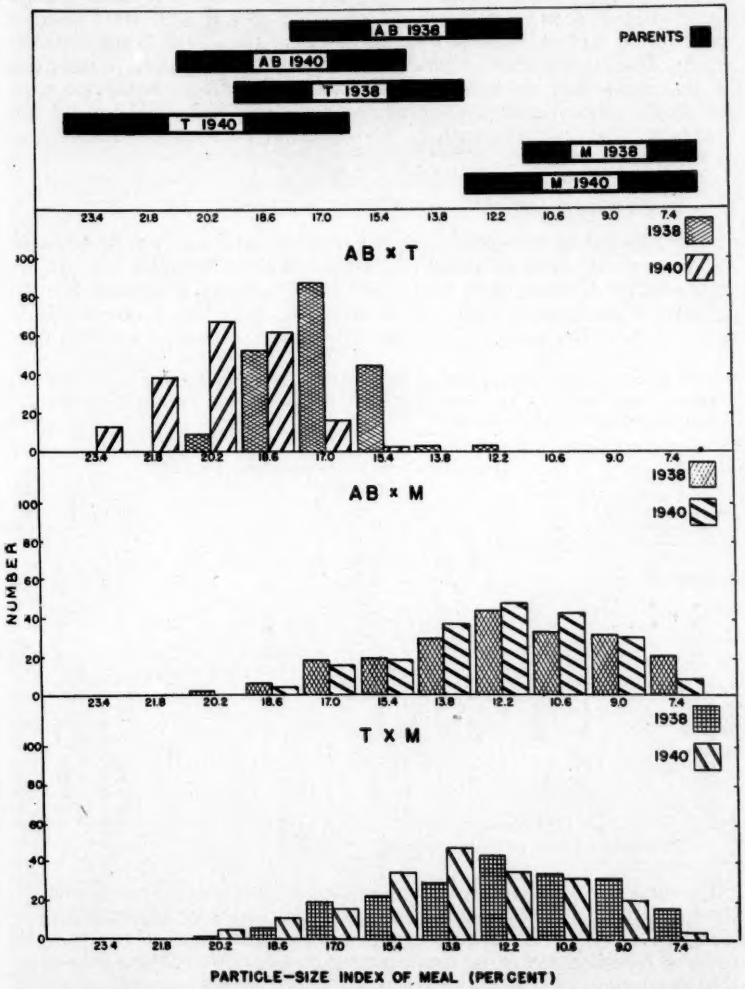

FIGURE 2.—Range in particle-size index of the parent varieties American Banner, Trumbull, and Michikof, and F_2 distribution of particle-size index of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses. Crops of 1938 and 1940.

Banner and Trumbull, respectively, indicates that these varieties grind into a relatively fine meal, whereas the variety Michikof, with an index of 8.8, produces a coarse meal.

The hybrids, considered as a whole, are intermediate to the parents in meal granulation with F_3 families varying in degree of particle fineness from the finer to the coarser grinding parent. The frequency distributions of particle-size index, for each of the 3 hybrid populations, suggest a normal curve. F_4 lines (216 and 322) were selected that varied widely in meal granulation and that bred comparatively true. The results show, therefore, that meal granulation is inherited in the same way as other quantitative characters; however, since parental recombinations reappeared many times in populations of 200, it appears that only a few genetic factors govern the mode of inheritance of meal granulation in the varieties studied.

Carotenoid Pigment Content

The amount of carotenoid pigments in wheat usually reflects color, an important characteristic of white flour. Samples of wheat, representing hybrid lines and parent rows, were analyzed for the amount of carotenoid pigments in order to study the mode of inheritance of this character. The data are shown in table 4 and figure 3.

Table 4.—*Frequency distribution of carotenoid pigments of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses and of the parent varieties*

Parent or cross	Generation	Year	Lines in class center for carotene in parts per million stated											Lines	Mean	Standard deviation
			1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5			
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*p. p. m.*	
American Banner	P_1	1938	----	----	6	11	5	----	----	----	----	----	----	----	22	1.90	0.15
Do	P_1	1940	----	----	8	13	1	----	----	----	----	----	----	----	22	1.84	.11
Trumbull	P_1	1938	----	----	2	13	7	----	----	----	----	----	----	----	22	1.93	.11
Do	P_1	1940	----	----	2	10	10	----	----	----	----	----	----	----	22	1.97	.14
Michikof	P_1	1938	----	----	----	8	10	4	----	----	----	----	----	----	22	2.06	.13
Do	P_1	1940	----	----	----	3	14	5	----	----	----	----	----	----	22	2.12	.12
AB × T	F_3	1938	----	28	78	73	16	5	----	----	----	----	----	----	200	1.80	.19
Do	F_4	1940	----	3	47	87	50	11	2	----	----	----	----	----	200	1.93	.18
AB × M	F_3	1938	2	15	26	34	69	40	10	2	2	----	----	----	200	2.04	.29
Do	F_4	1940	1	21	38	51	53	26	9	1	----	----	----	----	200	1.95	.27
T × M	F_3	1938	7	33	35	52	36	25	10	1	1	----	----	----	200	1.90	.32
Do	F_4	1940	1	19	25	37	41	39	24	10	4	----	----	----	200	2.09	.35
Pedigree No.:																	
289	F_4	1940	3	24	3	----	----	----	----	----	----	----	----	----	30	1.50	.09
277	F_4	1940	----	6	4	9	6	2	1	----	----	----	----	----	28	1.88	.27
228	F_4	1940	----	----	1	1	5	11	6	----	----	----	----	----	24	2.27	.20
299	F_4	1940	----	----	1	2	10	6	3	4	2	----	----	----	28	2.30	.31
380	F_4	1940	----	----	----	----	----	----	----	4	7	7	6	3	27	3.08	.25

The actual values for total carotenoid content in wheat shown in table 4 and figure 3 are somewhat lower than usually reported in the literature. There is disagreement among investigators as to the material best suited for the preparation of standards in the calibration of photoelectric instruments. In this study pure beta-carotene, which is not completely soluble in water-saturated butanol, was used.

The results show that the parental varieties do not differ greatly in carotenoid content; however, the variety, Michikof, is significantly

higher in the amount of yellow pigments than the varieties American Banner or Trumbull. In each cross, the F_2 distributions exceed the

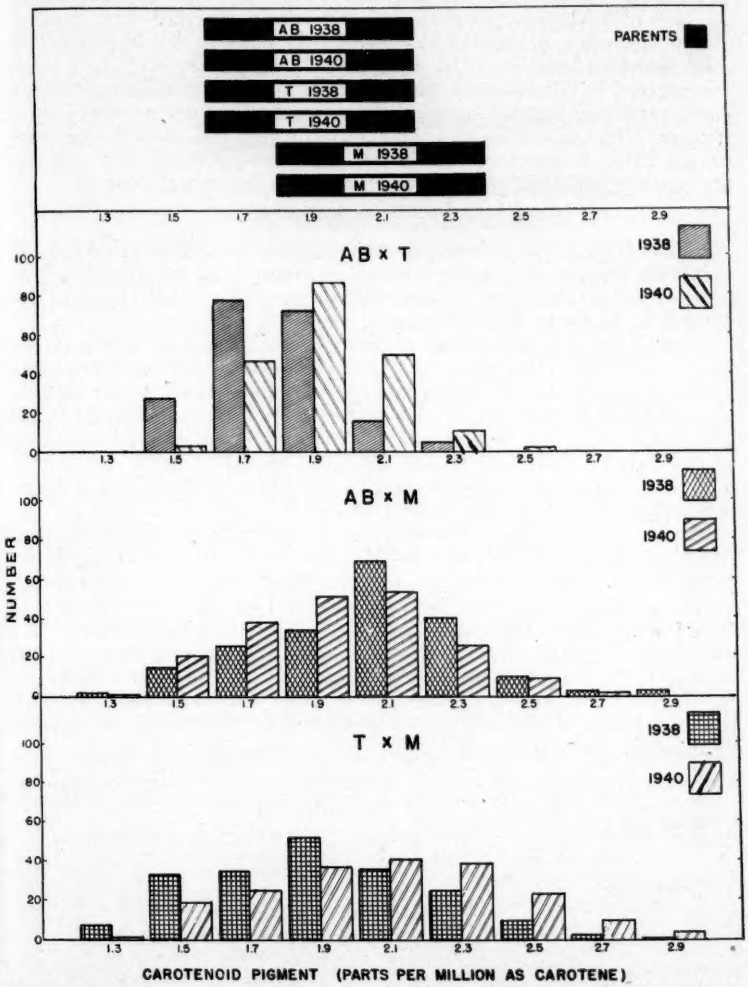

FIGURE 3.—Range in carotenoid pigment content of the parent varieties American Banner, Trumbull, and Michikof, and F_2 distribution of carotenoid pigment content of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses. Crops of 1938 and 1940.

range of the parent varieties, indicating transgressive segregation for carotenoid content. F_3 families as low as 1.3 parts per million and as

494656—42——2

high as 2.9 parts per million in carotene were recombined. The frequency distributions of carotene for the hybrid populations approach normal curves. F_4 lines were selected that varied from an average of 1.50 parts per million to 3.08 parts per million in carotene.

The sharp rise in variability between the parents and F_3 generation, and the recombination of lines beyond the range found in the parents (transgressive inheritance), indicates that the mode of inheritance of carotenoid pigment content in wheat is governed by several genetic factors. The data show that although the varieties used in this study varied little in carotenoid content, they are genotypically different and possess different pairs of genetic factors for this character.

Crude Protein

Crude-protein determinations were made on several hundred samples of wheat, representing hybrids and parent rows, in order to study the manner of inheritance of this character. The data obtained are presented in table 5 and figure 4.

A study of the inheritance of crude-protein content is difficult to make since this character is greatly influenced by environmental conditions. In the 1938 crop, the protein content of the 22 samples of American Banner wheat, all grown within an area of one-fourth acre, varied from 8.5 to 10.0 percent, and for the same year the samples of the variety Trumbull varied from 8.5 to 11.5 percent in protein. Also, the wheat harvested during the 1938 season contained a higher percentage of protein that that of the 1940 crop.

On the basis of the 2 years' data the average protein content of American Banner, Trumbull, and Michikof is 9.1, 9.6, and 10.6 percent, respectively. The differences between these values are statistically significant, indicating that the varieties used in this study are genetically different in crude-protein content. The hybrids, considered as a whole, are intermediate to the parents, with lines varying in percentage protein from the lower to the higher parent. F_4 families that varied significantly in protein content were selected. The data indicate that the mode of inheritance of crude-protein content is conditioned by multiple factors.

Table 5.—*Frequency distribution of percentage of protein of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses and of the parent varieties*

Parent or cross	Generation	Year	Lines in class center for protein content in percentage stated										Lines	Mean	Standard deviation
			7.8	8.3	8.8	9.3	9.8	10.3	10.8	11.3	11.8	12.3			
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	
American Banner	P_1	1938	----	----	7	9	6	----	----	----	----	----	22	9.3	0.32
Do	P_1	1940	----	6	8	5	2	1	----	----	----	----	22	8.9	.56
Trumbull	P_1	1938	----	----	1	6	9	4	1	1	----	----	22	9.9	.56
Do	P_1	1940	----	----	3	11	7	1	----	----	----	----	22	9.4	.39
Michikof	P_1	1938	----	----	----	----	2	9	5	3	3	----	22	10.8	.57
Do	P_1	1940	----	----	----	1	4	11	5	1	----	----	22	10.3	.45
AB × T	F_2	1938	----	3	43	100	44	8	2	----	----	----	200	9.3	.43
Do	F_4	1940	1	14	56	75	48	5	1	----	----	----	200	9.2	.49
AB × M	F_2	1938	----	----	4	23	60	51	36	20	3	3	200	10.2	.69
Do	F_4	1940	1	8	51	90	39	11	----	----	----	----	200	9.3	.47
T × M	F_2	1938	----	----	2	10	52	65	54	13	4	----	200	10.3	.56
Do	F_4	1940	----	----	5	40	86	51	17	1	----	----	200	9.9	.47
Pedigree No.:															
20	F_4	1940	----	----	11	14	5	----	----	----	----	----	30	9.2	.36
299	F_4	1940	----	----	----	1	8	12	4	2	1	----	28	10.3	.55
228	F_4	1940	----	----	----	----	----	----	9	6	5	4	24	11.4	.56

Kernel Weight

The 1,000-kernel weight of wheat, representing F_3 families and parent rows, was determined when grown under similar environmental

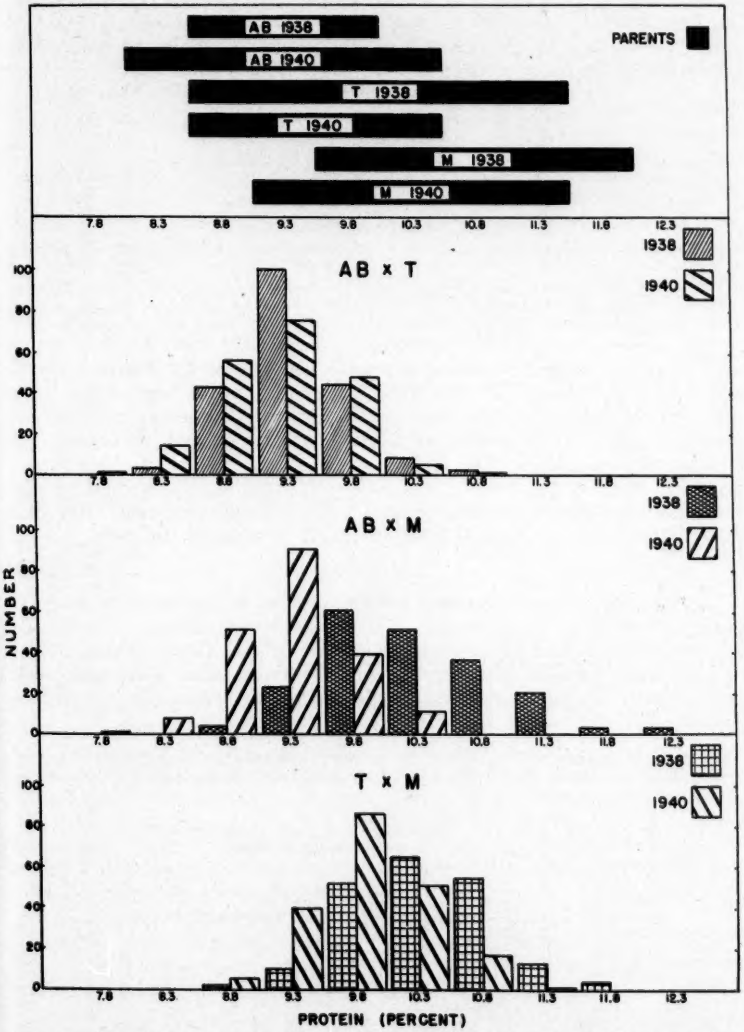

FIGURE 4.—Range in crude-protein content of the parent varieties American Banner, Trumbull, and Michikof, and F_2 distribution of crude-protein content of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses. Crops of 1938 and 1940.

conditions. The data for these and also for several selected F_4 families are presented in table 6.

TABLE 6.—*Frequency distribution of weight of 1,000 kernels of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses and of the parent varieties*

Parent or cross	Generation	Year	Lines in class center for 1,000-kernel weight in number of grams stated										Lines	Mean	Standard deviation
			25	27	29	31	33	35	37	39	41	43			
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Gms.*	
American Banner	P_1	1938	----	----	1	2	5	9	4	1	----	----	22	34.6	2.37
Do	P_1	1940	----	----	----	----	----	3	11	7	1	----	22	37.5	1.54
Trumbull	P_1	1938	----	----	----	----	5	11	6	----	----	----	22	35.1	1.28
Do	P_1	1940	----	----	----	1	1	12	8	----	----	----	22	35.4	1.50
Michikof	P_1	1938	----	----	1	11	9	1	----	----	----	----	22	31.6	1.27
Do	P_1	1940	----	----	1	8	12	1	----	----	----	----	22	32.2	1.34
AB × T	F_3	1938	----	----	4	9	21	36	56	42	25	7	200	36.9	3.06
Do	F_4	1940	----	----	----	2	18	52	82	39	7	----	200	36.6	2.00
AB × M	F_3	1938	----	----	4	37	47	48	47	16	1	----	200	34.5	2.60
Do	F_4	1940	----	----	5	31	53	64	36	10	1	----	200	34.3	2.37
T × M	F_3	1938	----	----	3	15	51	82	37	11	----	1	200	35.7	2.15
Do	F_4	1940	----	----	7	46	78	49	19	1	----	----	200	34.3	2.02
Pedigree No.:															
299	F_4	1940	5	8	9	6	----	----	----	----	----	----	28	28.2	2.04
216	F_4	1940	----	----	1	14	11	3	----	----	----	----	29	32.1	1.52
76	F_4	1940	----	----	----	3	11	10	2	----	----	----	26	33.8	1.62
5	F_4	1940	----	----	----	----	1	10	13	3	1	----	28	36.5	1.82

The kernel weight of wheat is greatly influenced by environmental conditions as indicated by the wide range found in the parent varieties. The results show that the varieties American Banner and Trumbull possess large kernels while the kernels of the variety Michikof are considerably smaller. The hybrid populations, as a whole, are intermediate to the parents in kernel size. F_4 families that varied widely in kernel weight were recombined. These data suggest that the inheritance of kernel weight is conditioned by multiple factors.

TEST WEIGHT

Test weight is an important component of wheat quality since it generally reflects flour yield. To study its mode of inheritance test-weight determinations were made on wheat from hybrid populations and parent rows grown under comparable environmental conditions. The data, arranged in frequency distributions, are shown in table 7.

TABLE 7.—*Frequency distribution of test weight of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses and of the parent varieties*

Parent or cross	Generation	Year	Lines in class center for test weight in number of pounds stated										Lines	Mean	Standard deviation
			54.5	55.5	56.5	57.5	58.5	59.5	60.5	61.5	62.5	63.5			
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Lb.*	
American Banner	P_1	1938	2	4	3	9	3	1	----	----	----	----	22	56.9	1.24
Do	P_1	1940	----	----	3	7	9	3	----	----	----	----	22	58.1	.92
Trumbull	P_1	1938	----	1	1	8	5	6	1	----	----	----	22	58.8	.83
Do	P_1	1940	----	----	----	3	1	12	6	----	----	----	22	59.5	.95
Michikof	P_1	1938	----	----	1	2	2	7	7	3	----	----	22	60.0	1.29
Do	P_1	1940	----	----	----	----	1	6	10	2	3	----	22	60.5	1.07
AB × T	F_3	1938	2	6	22	44	79	43	4	----	----	----	200	58.2	1.13
Do	F_4	1940	2	13	34	43	58	47	3	----	----	----	200	58.0	1.29
AB × M	F_3	1938	----	----	8	35	60	67	30	----	----	----	200	58.9	1.60
Do	F_4	1940	----	----	----	1	5	37	107	48	2	----	200	60.5	.78
T × M	F_3	1938	----	----	1	6	27	68	80	15	3	----	200	59.9	.99
Do	F_4	1940	----	----	----	----	1	12	74	95	18	----	200	61.1	.76
Pedigree No.:															
76	F_4	1940	5	5	10	5	1	----	----	----	----	----	26	56.2	1.12
5	F_4	1940	----	----	----	9	13	6	----	----	----	----	28	58.4	.74
289	F_4	1940	----	----	----	----	1	9	17	2	1	----	30	60.3	.78
322	F_4	1940	----	----	----	----	----	1	10	16	3	----	30	61.2	.70
277	F_4	1940	----	----	----	----	----	----	----	9	12	7	28	62.4	.78

Comparing the data of the two seasons, it is evident that the wheat of the 1938 crop was lower in test weight than that of the 1940 crop. The average test weights, for the 2 years, of American Banner, Trumbull, and Michikof, are 57.5, 59.2, and 60.2 pounds, respectively. The F_2 distributions of test weight, for each of the 3 hybrid populations, suggest normal curves. F_4 families varying from 56.2 to 62.4 pounds in test weight were selected. The data indicate, therefore, that test weight is a quantitative character governed to some extent by several genetic factors.

Cold Resistance

In studying the inheritance of cold resistance, F_3 and F_4 lines, together with their parents, were subjected to artificially controlled freezing tests during the winters of 1938–39 and 1939–40. The data obtained are presented in table 8.

Table 8.—*Frequency distribution of cold resistance of hybrids from American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses and of the parent varieties, as determined by controlled freezing tests*

Parent or cross	Generation	Lines in class center for estimated winter survival in percentage stated									Lines	Mean	Standard deviation
		17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5			
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	
American Banner	P_1	19	31								50	20.6	2.42
Trumbull	P_1	1	31	18							50	24.2	2.61
Michikof	P_1						1	17	29	3	50	50.9	3.11
AB × T	F_2	35	136	29							200	22.4	2.83
AB × M	F_2	2	14	37	51	53	32	10	1		200	34.5	6.78
T × M	F_2		1	11	25	65	57	28	13		200	38.0	6.26
Pedigree No.:													
509	F_4	16	3								19	18.3	1.88
380	F_4	3	11	10	3						27	24.9	4.27
219	F_4		2	12	9	5					28	30.5	4.42
635	F_4			1	12	5	3				21	34.9	4.09
339	F_4					1	5	16	4		26	46.9	4.06

The results show an average estimated winter survival of 20.6, 24.2, and 50.9 percent for the parental varieties American Banner, Trumbull, and Michikof, respectively. F_3 families varied in cold resistance from that of the tender to that of the hardier parent, with the majority of the lines intermediate to the parents. Some F_4 lines were recovered that were as susceptible to frost injury as the nonhardy variety American Banner, while others were almost as hardy as Michikof.

The increase in the variability between the parents and the F_3 generation indicates the segregation of genetic factors. The results indicate, therefore, that cold resistance is a quantitative character conditioned by several genetic factors. These results confirm the findings of other investigators, especially those of Hayes and Aamodt (*26*), Nilsson-Ehle (*33*), Quisenberry (*34*), and Worzella (*44*).

Morphological Characters

Since the parental varieties varied in several morphological characters, records were made of the colors of the straw, coleoptile, glume, and kernels of the hybrid materials. On the basis of F_3 breeding behavior, the families were classified as homozygous dominant,

heterozygous, or homozygous recessive. Since the development of straw color was influenced considerably by environmental conditions, some difficulty was experienced in distinguishing between bleached purple and white culms. Consequently, the homozygous dominant and heterozygous families for straw color were combined. The results were summarized and the F_2 segregation of the morphological characters in three crosses is given in table 9.

It will be noted from the data in table 9 that the hybrids segregated

TABLE 9.—*F_2 segregation of morphological characters in American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses*

Parents and characters	Ratio	Number of families in class indicated			χ^2	P
		Homozygous dominant	Heterozygous	Homozygous recessive		
American Banner × Trumbull:						
Brown and white glume	Observed	49	102	49		
	Calculated (1:2:1)	50	100	50	0.08	0.96
Red and white kernel	Observed	53	97	50		
	Calculated (1:2:1)	50	100	50	.27	.89
Purple and green coleoptile	Observed	50	107	43		
	Calculated (1:2:1)	50	100	50	1.47	.48
Purple and white straw	Observed	157		43		
	Calculated (3:1)	150		50	1.31	.26
American Banner × Michikof:						
Brown and white glume	Observed	50	105	45		
	Calculated (1:2:1)	50	100	50	.75	.73
Red and white kernel	Observed	196		4		
	Calculated 63:1)	196.9		3.1	.27	.68
Trumbull × Michikof:						
Purple and green coleoptile	Observed	47	103	50		
	Calculated (1:2:1)	50	100	50	.27	.89
Purple and white straw	Observed	150		50		
	Calculated (3:1)	150		50	0	1.00

in a 1:2:1 ratio for glume, coleoptile, and kernel color, and a 3:1 ratio for straw color. These ratios indicate a single-factor difference for each of the characters in the crosses studied. Also, in the American Banner × Michikof cross, three pairs of factors are involved in the inheritance of kernel color.

INTERANNUAL CORRELATIONS

Interannual correlation coefficients were calculated for the components of wheat quality in order to learn the extent to which the hybrids reacted in the same way in different seasons. These data are reported in table 10.

It will be noted that highly significant positive interannual correlation coefficients were obtained for the components of quality studied. Since the soft wheat varieties, American Banner and Trumbull, vary slightly in quality, hybrids from this cross show correlation coefficients of lower magnitude than those obtained on the more diverse hybrids from crosses involving hard and soft wheats.

The high coefficients of correlation for gluten strength, granulation, and carotenoid content, especially in the American Banner × Michikof and Trumbull × Michikof crosses, indicate that the hybrids reacted in much the same way in the two seasons. The magnitude of the interannual correlations for protein, 1,000-kernel weight, and test weight, although highly significant, is rather low, indicating that environmental conditions greatly influence the expression of these characters.

TABLE 10.—*Interannual correlation coefficients of components of wheat quality of 200 hybrids from each of American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses grown during 1938 and 1940*

Components of quality	Correlation coefficients on the data obtained from the 1938 and 1940 crops in[1]—		
	American Banner × Trumbull	American Banner × Michikof	Trumbull × Michikof
Gluten strength	0.49	0.79	0.77
Granulation	.47	.84	.89
Carotenoid content	.28	.76	.82
Protein	.30	.35	.37
1,000-kernel weight	.47	.65	.53
Test weight	.24	.42	.49

[1] A correlation coefficient of 0.181 is at the 1-percent level of significance.

The consistency and magnitude of the interannual correlation coefficients for each of the three crosses provides further evidence that the variance observed in the characters gluten strength, granulation, carotenoid content, protein, kernel weight, and test weight in wheat is, in part, genetic in nature.

INTERRELATIONSHIP BETWEEN CHARACTERS

Many wheat investigators are of the opinion that associations exist between certain components of quality and also between certain of these and winter hardiness. For example, (1) hard vitreous wheats are regarded as being high in protein content and possessing a strong gluten, while soft starchy kernels are considered as being low in protein and weak in gluten strength, (2) white wheats are believed to possess weaker gluten than redkernel wheats- and (3) soft wheats are regarded as less winter hardy than hard wheats. These relationships are based, for the most part, on observations and experiments conducted on a series of varieties or selected hybrid strains. Associations between characters obtained from such studies would be affected by the wheats chosen, and would not necessarily reflect the natural relationships present in randomly selected hybrid populations.

For the present investigations 200 hybrid families from each cross, selected at random, were used. Obviously, such a sample represents a population of wheats in which the recombination of characters was governed by the laws of heredity. In determining the relationships between the 11 characters studied, the data were subjected to correlation, chi-square, and linkage analysis.

CORRELATION AND CHI-SQUARE ANALYSIS

To measure the degree of association, if any, the method of correlation was used for the quantitative characters, while the chi-square test was used where qualitative characters were involved. Since the hybrids of the three crosses represent different populations, correlation and chi-square analysis were calculated separately for each cross. The data, indicating the interrelationship of components of quality, cold resistance, and several morphological characters of wheat hybrids from three crosses, are reported in tables 11, 12, and 13.

The intercharacter correlations in tables 11, 12, and 13, indicate

very little, if any, association between cold resistance and the components of quality studied. Although several of the correlation coefficients are statistically significant, they are rather small and inconsistent in each of the three crosses and for the 2 crop years. The morphological characters kernel color, glume color, coleoptile color, and straw color, are inherited independently of cold resistance.

TABLE 11.—*Relations among components of quality, cold resistance, and morphological characters of 200 wheat hybrids from American Banner × Trumbull cross grown during 1938 and 1940, expressed by correlation coefficients and chi-square probabilities* [1]

Characters	Crop of—	Association between characters indicated [2]: Cold resistance	Gluten strength	Granulation	Carotenoid content	Protein	1,000-kernel weight	Test weight	Kernel color	Glume color	Coleoptile color
Gluten strength	1938	0.07									
	1940	−.12									
Granulation	1938	.11	0.07								
	1940	.06	.26**								
Carotenoid content	1938	−.04	−.17*	0.38**							
	1940	−.10	−.02	−.01							
Protein	1938	.09	.13	−.08	−0.08						
	1940	.08	.28**	−.04	−.11						
1,000-kernel weight	1938	.06	.12	−.30**	−.53**	−0.10					
	1940	−.02	.04	−.06	−.39**	.03					
Test weight	1938	.12	.02	−.17*	−.33**	−.12	0.42**				
	1940	.10	−.02	−.23**	−.17*	−.10	.17*				
Kernel color	1938	71	54	13	76	<1**	61	61			
	1940	71	85	40	10	<1**	79	56			
Glume color	1938	24	<1**	70	49	77	6	46	78		
	1940	24	2*	30	97	20	81	75	53		
Coleoptile color	1938	80	28	95	77	82	15	60	83	87	
	1940	80	75	74	50	78	60	86	26	72	
Straw color	1938	80	28	95	77	82	15	60	83	87	0**
	1940	80	75	74	50	78	60	86	26	72	0**

[1] Percentage probability for chi-square test.
[2] *Odds at least 19 to 1; **odds at least 99 to 1.

TABLE 12.—*Relations among components of quality, cold resistance, and morphological characters of 200 wheat hybrids from American Banner × Michikof cross grown during 1938 and 1940, expressed by correlation coefficients and chi-square probabilities* [1]

Characters	Crop of—	Association between characters indicated [2]: Cold resistance	Gluten strength	Granulation	Carotenoid content	Protein	1,000-kernel weight	Test weight
Gluten strength	1938	0.10						
	1940	.08						
Granulation	1938	−.17*	0.01					
	1940	−.09	.03					
Carotenoid content	1938	−.04	−.08	0.16*				
	1940	.03	.14*	.17*				
Protein	1938	−.08	.24**	.16*	0			
	1940	−.15*	.26**	−.01	.02			
1,000-kernel weight	1938	.22**	.15*	−.20**	−.10	0.01		
	1940	.02	.02	−.10	−.17*	.16*		
Test weight	1938	.05	.18*	−.12	−.16*	.10	0.33**	
	1940	−.10	.05	−.04	−.06	.02	.15*	
Glume color	1938	98	<1**	47	73	73	44	27
	1940	98	<1**	91	85	62	40	84

[1] Percentage probability for chi-square test.
[2] *Odds at least 19 to 1; **odds at least 99 to 1.

TABLE 13.—*Relations among components of quality, cold resistance, and morphological characters of 200 wheat hybrids from Trumbull × Michikof cross grown during 1938 and 1940, expressed by correlation coefficients and chi-square probabilities* [1]

Characters	Crop of—	Association between characters indicated [2]							
		Cold resistance	Gluten strength	Granulation	Carotenoid content	Protein	1,000-kernel weight	Test weight	Coleoptile color
Gluten strength	1938	−0.17*							
	1940	−.07							
Granulation	1938	−.10	−0.07						
	1940	−.06	−.05						
Carotenoid content	1938	−.10	−.02	0.33**					
	1940	−.13	.14*	.13					
Protein	1938	−.22**	.03	.07	−0.01				
	1940	.07	.02	−.04	−.13				
1,000-kernel weight	1938	−.11	−.07	.09	−.17*	−0.09			
	1940	.04	.16*	.15*	−.13	.06			
Test weight	1938	.09	−.04	−.09	−.14*	−.09	0.31**		
	1940	−.06	.13	−.28**	−.16*	.27**	.11		
Coleoptile color	1938	41	93	38	58	2*	55	19	
	1940	41	34	70	53	37	59	61	
Straw color	1938	41	93	38	58	2*	55	19	0**
	1940	41	34	70	53	37	59	61	0**

[1] Percentage probability for chi-square test.
[2] *Odds at least 19 to 1; **odds at least 99 to 1.

The data indicate that in these hybrid populations, at least, the character gluten strength appears to be inherited independently of the other components of quality. However, highly significant correlation coefficients were obtained between gluten strength and protein content in the American Banner × Michikof cross (table 12). The results indicate that the genes controlling kernel color, coleoptile color, and straw color were not linked with those for gluten strength.

The positive correlation coefficients obtained between granulation and carotenoid content suggest that these characters are associated. It is well known, however, that granulation or fineness of the wheat meal greatly influences the extraction of the carotenoid pigments. It appears probable, therefore, that the correlations obtained between granulation and carotenoid content are due to the limitations of the grinding methods in the preparation of meal of comparable fineness, rather than to genetic linkage. Granulation appears to be correlated negatively with test weight, however; the coefficients are either small or nonsignificant. Very little, if any, relationship was found between granulation and the other quality factors and morphological characters.

Carotenoid pigment content was found to be correlated negatively with 1,000-kernel weight and test weight. Also, 1,000-kernel weight was correlated positively with test weight. It appears, therefore, that hybrids possessing large kernels that are high in test weight, tend to be lower in carotenoid content and vice versa. The relationships between these characters, however, are not close, since most of the coefficients are low in magnitude and others are not significant.

Chi-square analysis of the data reveals that the genes for the following characters are linked: (1) gluten strength and glume color, (2) protein content and kernel color, and (3) coleoptile color and straw color.

Genetic data reported in table 9 indicate that the inheritance of coleoptile and straw color is monogenic. Pedigree records reveal that, without exception, all of the hybrids were classified as either purple coleoptile with purple straw or green coleoptile with white straw. If 2 independent pairs of genetic factors were involved, one would expect a double recessive combination once out of every 16 F_3 lines. None were found in the 400 families examined. It appears that the inheritance of coleoptile and straw color is governed either by 2 pairs of genetic factors closely linked, or by the same pair of genes.

Linkage of Gluten Strength and Glume Color

Data reported in tables 11 and 12 show an association between the quantitative character gluten strength and the qualitative character glume color. This relation suggests a genetic linkage between a single pair of genes for glume color and one or more pairs of genes for gluten strength. Since in the American Banner × Trumbull cross, the inheritance of both characters, glume color and gluten strength, was found to be monogenic (table 2, fig. 1), it was possible to calculate their linkage intensity. The percentage crossing over was calculated on the F_2 segregation by the product method formula. The data are shown in table 14.

Table 14.—*Linkage relation between glume color (Gc gc) and gluten strength (G g) in American Banner (Gc g) (Gc g) × Trumbull (gc G) (gc G) cross*

Item	Crop year	Number of families having phenotype—				χ^2	P
		Gc G	*Gc g*	*gc G*	*gc g*		
Actual	1938	102	49	44	5		
Calculated, 30.5 percent crossing over	1938	104.6	45.4	45.4	4.6	0.42	0.85
Actual	1940	110	41	42	7		
Calculated, 38.8 percent crossing over	1940	107.5	42.5	42.5	7.5	.15	.93

It will be noted that approximately 30.5 percent and 38.8 percent crossing over was observed between the pair of genes for glume color and that governing the inheritance of gluten strength for the 1938 and 1940 crop years, respectively. The results of the 2 years agree rather closely, considering that these characters appeared in the repulsion phase and that only 200 hybrids were used.

Linkage of Protein Content and Kernel Color

In table 11 it was noted that, in the hybrids originating from the American Banner × Trumbull cross, the characters protein content and kernel color were associated. The protein data reported in table 5 and figure 4 suggest that probably a single pair of genetic factors controls the inheritance of this character in the American Banner × Trumbull cross. Accordingly, arbitrary classes for protein content were set up, and the hybrids classified into three groups approaching a 1:2:1 ratio. Table 15 reports the F_2 distribution and percentage crossing over for protein content and kernel color in the American Banner × Trumbull cross.

TABLE 15.—*Linkage relation between kernel color (R r) and protein content (N n) in American Banner (r n) (r n) × Trumbull (R N) (R N) cross*

Item	Crop year	Number of families having phenotype—				χ^2	P
		R N	*R n*	*r N*	*r n*		
Actual	1938	130	20	24	26		
Calculated, 25.7 percent crossing over	1938	127.6	22.4	22.4	27.6	0.51	0.81
Actual	1940	127	23	28	22		
Calculated, 30.9 percent crossing over	1940	123.9	26.1	26.1	23.9	.79	.71

The results in table 15 show that about 25.7 percent and 30.9 percent crossing over occurred between the pair of genes for protein content and that for kernel color for the 1938 and 1940 crop years, respectively. The results of the 2 years agree closely, considering that protein content is greatly influenced by environmental conditions.

SUMMARY

Experiments were conducted on 600 F_3 and F_4 hybrids, and 66 parent rows originating from a triangular cross of American Banner, Trumbull, and Michikof wheats, grown at Lafayette, Ind., in 1937–38 and repeated in 1939–40.

The inheritance and interrelationship of components of quality, cold resistance, and several morphological characters were studied in randomly selected hybrid populations.

The mode of inheritance of gluten strength, granulation, carotenoid content, crude protein, kernel weight, test weight, and cold resistance was found to be quantitative and governed by several genetic factors. In the hybrids originating from the American Banner × Trumbull cross, the inheritance of gluten strength and protein content appeared to be monogenic.

Single genetic-factor differences were found to govern the mode of inheritance of the characters glume, coleoptile, and straw color. The inheritance of kernel color was governed by one and three pairs of genes in the crosses studied.

Highly significant interannual correlation coefficients were obtained for the components of quality studied. The consistency and high magnitude of the correlations provide further evidence that the variance observed in the characters gluten strength, granulation, carotenoid content, protein content, kernel weight, and test weight in wheat is, in part, genetic in nature.

Intercharacter correlations indicate very little, if any, association between cold resistance and the components of quality studied.

Few relationships were found between components of wheat quality in hybrid material selected at random. In general, for most of the characters, the coefficients were low in magnitude or not significant. Gluten strength and granulation were inherited independently of the other components of quality. However, granulation was correlated negatively with test weight. Carotenoid content was correlated negatively with kernel weight and test weight, while kernel weight was correlated positively with test weight.

Genetic linkage was found between the genes for the following characters: (1) gluten strength and glume color, (2) protein content and kernel color, and (3) coleoptile color and straw color.

LITERATURE CITED

(1) AAMODT, O. S., and TORRIE, J. H.
1934. A SIMPLE METHOD FOR DETERMINING THE RELATIVE WEIGHT PER BUSHEL OF THE GRAIN FROM INDIVIDUAL WHEAT PLANTS. Canad. Jour. Res. 11: 589–593.

(2) ——— and TORRIE, J. H.
1935. STUDIES ON THE INHERITANCE OF AND THE RELATION BETWEEN KERNEL TEXTURE AND PROTEIN CONTENT IN SEVERAL SPRING WHEAT CROSSES. Canad. Jour. Res. (C) 13: 202–219.

(3) ——— TORRIE, J. H., and WILSON, A.
1935. STUDIES OF THE INHERITANCE OF AND THE RELATIONSHIPS BETWEEN KERNEL TEXTURE, GRAIN YIELD, AND TILLER-SURVIVAL IN CROSSES BETWEEN REWARD AND MILTURUM SPRING WHEATS. Amer. Soc. Agron. Jour. 27: 456–466.

(4) AKERMAN, Å.
1922. IAKTTAGELSER RÖRANDE HÖSTSÄDENS OVERVINTRING VINTERN 1921–1922, [THE RESISTANCE SHOWN BY NUMEROUS VARIETIES OF WHEAT AT SVALÖF DURING THE WINTER 1921–1922.] Sveriges Utsädesför. Tidskr. 32: 252–266, illus. [Abstract in Internatl. Rev. Sci. and Pract. Agr. (Rome) (n. s.) 1: 350–353. 1923.]

(5) ALABOUVETTE, M.
1937. CONTRIBUTION A L'ÉTUDE DU CARACTÈRE "FORCE" DU BLÉ. Acad. d'Agr. de France, Compt. Rend. 23: 379–383.

(6) AMERICAN ASSOCIATION OF CEREAL CHEMISTS.
1941. CEREAL LABORATORY METHODS. Ed. 4, 264 pp., illus. Lincoln, Nebr.

(7) AUSEMUS, E. R.
1934. CORRELATED INHERITANCE OF REACTION TO DISEASES AND OF CERTAIN BOTANICAL CHARACTERS IN TRIANGULAR WHEAT CROSSES. Jour. Agr. Res. 48: 31–57, illus.

(8) ——— MARKLEY, M. C., BAILEY, C. H., and HAYES, H. K.
1938. QUALITY STUDIES IN THE WHEAT-BREEDING PROGRAM AT THE MINNESOTA AGRICULTURAL EXPERIMENT STATION. Jour. Agr. Res. 56: 453–464.

(9) BIFFEN, R. H.
1905. MENDEL'S LAWS OF INHERITANCE AND WHEAT BREEDING. Jour. Agr. Sci. [England] 1: [4]–48, illus.

(10) ———
1908. ON THE INHERITANCE OF STRENGTH IN WHEAT. Jour. Agr. Sci. [England] 3: [86]–101, illus.

(11) BINNINGTON, D. S., and GEDDES, W. F.
1939. A RAPID METHOD FOR THE DETERMINATION OF WHEAT AND FLOUR PIGMENTS. Cereal Chem. 16: 252–262, illus.

(12) BRYAN, W. E., and PRESSLEY, E. H.
1920. PLANT BREEDING. Ariz. Agr. Expt. Sta. Ann. Rpt. 1920: [480]–483.

(13) ——— and PRESSLEY, E. H.
1933. HARD GRAIN TEXTURE OF WHEAT IN MECHANICAL MIXTURES AND IN CROSSES. Amer. Soc. Agron. Jour. 25: 64–69.

(14) CHURCHWARD, J. G.
1938. STUDIES ON PHYSIOLOGIC SPECIALISATION OF THE ORGANISMS CAUSING BUNT IN WHEAT, AND THE GENETICS OF RESISTANCE TO THIS AND CERTAIN OTHER WHEAT DISEASES. PART II. GENETICAL STUDIES. Roy. Soc. N. S. Wales, Jour. and Proc. 71: 547–590, illus.

(15) CLARK, J. A.
1926. BREEDING WHEAT FOR HIGH PROTEIN CONTENT. Amer. Soc. Agron. Jour. 18: 648–661, illus.

(16) ——— Florell, V. H., and Hooker, J. R.
1928. INHERITANCE OF AWNEDNESS, YIELD, AND QUALITY IN CROSSES BETWEEN BOBS, HARD FEDERATION, AND PROPO WHEATS AT DAVIS, CALIFORNIA. U. S. Dept. Agr. Tech. Bul. 39, 40 pp., illus.
(17) ——— and Hooker, J. R.
1926. SEGREGATION AND CORRELATED INHERITANCE IN MARQUIS AND HARD FEDERATION CROSSES, WITH FACTORS FOR YIELD AND QUALITY OF SPRING WHEAT IN MONTANA. U. S. Dept. Agr. Dept. Bul. 1403, 71 pp., illus.
(18) ——— and Quisenberry, K. S.
1929. INHERITANCE OF YIELD AND PROTEIN CONTENT IN CROSSES OF MARQUIS AND KOTA SPRING WHEATS GROWN IN MONTANA. Jour. Agr. Res. 38: 205–217.
(19) ——— and Smith, R. W.
1928. INHERITANCE IN NODAK AND KAHLA DURUM WHEAT CROSSES FOR RUST RESISTANCE, YIELD, AND QUALITY AT DICKINSON, NORTH DAKOTA. Amer. Soc. Agron. Jour. 20: 1297–1304.
(20) Cutler, G. H., and Worzella, W. W.
1933. THE WHEAT-MEAL FERMENTATION TIME TEST OF "QUALITY" IN WHEAT AS ADAPTED FOR SMALL PLANT BREEDING SAMPLES. Cereal Chem. 10: 250–262, illus.
(21) ——— and Worzella, W. W.
1936. THE WHEAT MEAL FERMENTATION TIME TEST FOR MEASURING QUALITY IN WHEAT. Ind. Agr. Expt. Sta. Cir. 218, 14 pp., illus.
(22) Freeman, G. F.
1918. PRODUCING BREAD-MAKING WHEATS FOR WARM CLIMATES. Jour. Hered. 9: 211–226, illus.
(23) Goulden, C. H., Geddes, W. F., and Whiteside, A. G. O.
1934. A STUDY OF CAROTENE CONTENT AND OTHER QUALITY CHARACTERS IN A SERIES OF HYBRID WHEATS. Cereal Chem. 11: 557–566, illus.
(24) Harrington, J. B.
1922. THE MODE OF INHERITANCE OF CERTAIN CHARACTERS IN WHEAT. Sci. Agr. 2: 319–324.
(25) Hayes, H. K.
1923. INHERITANCE OF KERNEL AND SPIKE CHARACTERS IN CROSSES BETWEEN VARIETIES OF TRITICUM VULGARE. Minn. Univ. Studies Biol. Sci. 4: [163]–183.
(26) ——— and Aamodt, O. S.
1927. INHERITANCE OF WINTER HARDINESS AND GROWTH HABIT IN CROSSES OF MARQUIS WITH MINHARDI AND MINTURKI WHEATS. Jour. Agr. Res. 35: 223–236.
(27) ——— Immer, F. R., and Bailey, C. H.
1929. CORRELATION STUDIES WITH DIVERSE STRAINS OF SPRING AND WINTER WHEATS, WITH PARTICULAR REFERENCE TO INHERITANCE OF QUALITY. Cereal Chem. 6: 85–96.
(28) Howard, A., and Howard, G. L. C.
1912. ON THE INHERITANCE OF SOME CHARACTERS IN WHEAT. India Dept. Agr. Mem., Bot. Ser., 5: 1–[47], illus.
(29) Jasnowski, S.
1934. O DZIEDZICZENIU CIĘZARU ZIARNA U TRITICUM VULGARE L. [ON THE INHERITANCE OF WEIGHT OF GRAINS IN T. VULGARE L.] Roczn. Nauk Rol. 33: 59–68. (Imp. Bur. Plant Genet. Plant Breeding Abs. 5, Abst. 654, p. 217. 1935.)
(30) ———.
1935. O DZIEDZICZENIU NIEKTÓRYCH CECHKŁOSA PSZENICY. [ON THE INHERITANCE OF SOME CHARACTERS OF THE EAR OF WHEAT.] Bul. Acad. Polon. Sci. Lettres, Cracovie, 119–218. (Imp. Bur. Plant Genet. Plant Breeding Abs. 6, Abs. 844, p. 263. 1936.)
(31) Markley, M. C.
1937. VARIABILITY IN CAROTENOID PIGMENT CONTENT OF INDIVIDUAL PLANTS OF TRITICUM VULGARE AND TRITICUM DURUM. Cereal Chem. 14: 400–409, illus.
(32) Meneret, M. G.
1937. OBSERVATIONS SUR L' HÉRÉDITÉ DU CHARACTÈRE "QUALITÉ" DU BLÉ. Acad. d'Agr. de France, Compt. Rend. 23: 742–747, illus.

(33) Nilsson-Ehle, H.
1912. zur kenntnis der erblichkeitsverhältnisse der eigenschaft winterfestigkeit beim weizen. Ztschr. Pflanzenzücht. 1: 3–12.
(34) Quisenberry, K. S.
1931. inheritance of winterhardiness, growth habit, and stem-rust reaction in crosses between minhardi winter and h–44 spring wheats. U. S. Dept. Agr. Tech. Bul. 218, 46 pp., illus.
(35) ——— and Clark, J. A.
1929. breeding hard red winter wheats for winter hardiness and high yield. U. S. Dept. Agr. Tech. Bul. 136, 28 pp.
(36) Rosenstiel, K. V.
1934. untersuchungen zur genetik der kleberqualität bei winterweizen. (Vorläufige Mitteilung.) Züchert 6: 261–262.
(37) Saunders, C. E.
1907. quality in wheat. part i. Canada Expt. Farms Bul. 57: [7]–36.
(38) ———
1909. the inheritance of "strength" in wheat. Jour. Agr. Sci. [England] 3: [218]–222.
(39) Torrie, J. H.
1936. inheritance studies of several qualitative and quantitative characters in spring wheat crosses between varieties relatively susceptible and resistant to drought. Canad. Jour. Res. (C) 14: 368–385, illus.
(40) Waldron, L. R., and Mangels, C. E.
1932. correlational and allied studies of the protein content, water absorption, loaf volume, and loaf weight of two series of hard red spring wheats. Jour. Agr. Res. 45: 209–231, illus.
(41) Whiteside, A. G. O.
1931. a study of the carotinoid pigments of wheat and flour with special reference to wheat varieties. Canada Dept. Agr. Bul. (n. s.) 154, 33 pp.
(42) ——— Edgar J., and Goulden, C. H.
1934. the influence of environment on the carotenoid content of hard red spring wheat. Cereal Chem. 11: 615–625, illus.
(43) Worzella, W. W.
1934. the inheritance of quality in trumbull and michikof varieties of winter wheat. Jour. Agr. Res. 49: 705–714.
(44) ———
1935. inheritance of cold resistance in winter wheat, with preliminary studies on the technic of artificial freezing tests. Jour. Agr. Res. 50: 625–635, illus.
(45) ——— and Cutler, G. H.
1935. carotenoid pigments in wheat with special reference to varieties and strains. Cereal Chem. 12: 708–713.
(46) ——— and Cutler, G. H.
1938. character analysis of winter wheat varieties. Amer. Soc. Agron. Jour. 30: 430–433.
(47) ——— and Cutler, G. H.
1939. a critical study of technique for measuring granulation in wheat meal. Jour. Agr. Res. 58: 329–341, illus.
(48) ——— and Cutler, G. H.
1941. factors affecting cold resistance in winter wheat. Amer. Soc. Agron. Jour. 33: 221–230, illus.
(49) Zinn, J.
1920. wheat investigations. i. pure lines. Maine Agr. Expt. Sta. Bul. 285, 48 pp. illus.

THE RESPONSE OF "CEASED" REACTORS IN BANG'S DISEASE TO REEXPOSURE [1]

By B. A. BEACH, *professor of veterinary science, Wisconsin Agricultural Experiment Station;* M. R. IRWIN, *cooperative agent, Bureau of Animal Industry, and professor of genetics, Wisconsin Agricultural Experiment Station;* and L. C. FERGUSON, *cooperative agent, Bureau of Animal Industry, and instructor in veterinary science and genetics, Wisconsin Agricultural Experiment Station*

INTRODUCTION

The results of observations on cattle under controlled conditions, and also in the field, agree in supporting the conclusion that infection with a virulent strain of *Brucella abortus* usually induces resistance to subsequent infection by the organism, presumably as the result of active immunization. This phenomenon has long been recognized by some workers—among them Birch and Gilman (*2*) [2] and Giltner (*9*)—and has been questioned by others, particularly insofar as the possibility of complete recovery from the infection is concerned. Complete recovery would imply elimination of the organism from the animal's body and a return of the agglutination titer of the serum to that of a noninfected individual.

The observations reported in this paper were made on cows whose sera, following induced infections, had lost their agglutinin titer for the infecting organism. These observations were made on two groups of animals, in two different herds consecutively assembled, over a period from August 1933 through July 1939. In a preceding article (*1*), the explanation was made that not all the animals to which the term "ceased" reactor is applied, had been definite reactors according to the accepted standard. Since the principal point of interest in this paper is the reaction of all previously infected individuals to a second exposure, the terminology for these animals is unchanged.

MATERIALS AND METHODS

The animals on which these observations were made were in two herds, as previously described (*1*).

Herd 1 originally consisted of 44 grade and purebred Holstein calves, and was assembled in 1926 as described by Hart and his coworkers (*11*). After the artificial exposure of 37 pregnant individuals in 1928, and subsequent observations on them for a period of 2 years following the exposure, 14 individuals were selected to constitute a part of herd 2. At that time the agglutinins for *Brucella abortus* had practically or entirely disappeared from the serum of each of these 14 animals, and they were therefore termed "ceased" reactors.

To these 14 cows from herd 1 were added 22 animals, mostly virgin heifers, to make up herd 2. It was thought that none of these 22 animals had had any previous contact with *Brucella abortus*, although

[1] Italic numbers in parentheses refer to Literature Cited, p. 531.

[2] Received for publication February 27, 1942. Contribution from the Department of Veterinary Science and the Department of Genetics (paper No. 281), Wisconsin Agricultural Experiment Station, and the Bureau of Animal Industry, U. S. Department of Agriculture.

it was later determined that one cow (No. 23A) had been in a herd which had suffered an outbreak of Bang's disease some years previously. The individuals in herd 2 were artifically exposed in September 1933 by instilling two drops of a heavy suspension of *Brucella abortus* into the eye of each.

Herd 3 was assembled in August 1934. This group consisted of 8 individuals (ceased reactors) from herd 2, and 28 virgin heifers which were purchased from herds free of Bang's disease. One of the ceased reactors developed acute mastitis early in the experiment and was removed. Other removals and substitutions were made, as will be described more fully later. All animals in the herd were artificially exposed to *Brucella abortus* in May 1937. Cultures of the same strains of the organism were used that had previously been found by Crawford and Beach (*6*) to possess a marked pathogenicity for susceptible cattle.

The serum of each of the animals in both of the herds used in these experiments (herds 2 and 3) was tested, usually monthly, often weekly, for its agglutinin titer against *Brucella abortus*. Further, at each parturition, the colostrum and either the placenta or the uterine fluid, or both, were tested for the presence of *Brucella abortus* by the usual method of inoculation of guinea pigs.

EXPERIMENTAL RESULTS

HERD 2

Data are presented in table 1 for the animals of herd 2. These include the pregnancies completed prior to the induced infection of all the animals of the herd in September 1933, and the subsequent abortion or normal calving of each individual. The first 14 individuals listed in the table were the so-called ceased reactors from herd 1. The others, with the possible exception of 23A as described above, presumably had had no previous contact with *Brucella abortus*.

TABLE 1.—*Results obtained with the animals of herd 2 prior to and following their artificial exposure* [1] *to Brucella abortus*

Cow No.	Preexposure period			Postexposure period				*Brucella abortus* recovered—		Agglutination reaction and time observed	
	Number of pregnancies completed		*Brucella abortus* recovered	Days pregnant—		Days from exposure to abortion or calving	Aborted	At calving or abortion	Following slaughter	Highest titer	Weeks following exposure
	Normal	Abortion		When exposed	When aborted or calved						
4	2	0	No	26	283	257	No	No	No	<1:25	20
5	1	0		87	152	65	Yes	Yes	No	1:200	10
8	2	0	No	[2]					No	[3] 1:100	17
12	2	0	No	97	283	186	No	No	No	1:25	19
17	2	0	No	30	279	249	No	No	No	<1:25	13
18	2	0	No	[4]							
28	2	0	No	78	287	209	No	No	No	1:50	19
31 [5]	1	0	No	79	279	200	No	No	No	<1:25	15
33	2	0	No	94	283	189	No	No		<1:25	19
35	2	0	No	73	287	214	No	No	No	1:25	19
36	2	0	[6]	82	281	199	No	No	No	1:50	15
38	2	0	No	70	279	209	No	No	No	1:100	15
40	2	0	No	89	283	194	No	No	No	<1:25	19

See footnotes at end of table.

TABLE 1.—*Results obtained with the animals of herd 2 prior to and following their artificial exposure* [1] *to Brucella abortus*—Continued

Cow No.	Preexposure period			Postexposure period				*Brucella abortus* recovered—		Agglutination reaction and time observed	
	Number of pregnancies completed		*Brucella abortus* recovered	Days pregnant—		Days from exposure to abortion or calving	Aborted	At calving or abortion	Following slaughter	Highest titer	Weeks following exposure
	Normal	Abortion		When exposed	When aborted or calved						
44	2	0	No	96	281	185	No	No	No	>1:25	15
5A	1	0	No	[4]							
6A	2	0	No	[4]							
7A	2	0	No	[4]							
23A	2	0	No	36	281	245	No	No		1:25	15
25A	2	0	No	45	211	166	Yes	Yes	Yes	1:400	15
29A	2	0	No	26	196	170	Yes	Yes	Yes	1:400	13
32A	2	0	No	46	280	234	No	No		1:100	10
58A				34	235	201	Yes	No		1:50	8
61A				56	164	108	Yes	Yes	Yes	1:400	15
63A				77	287	364	No	No		1:25	4
1B	2	0	No	89	181	92	Yes	Yes	No	1:200	13
2B	2	0	No	49	164	115	Yes	No	No	1:25	4
3B	2	0	No	57	169	112	Yes	Yes	Yes	1:100	15
9B	1	0	No	7	278	271	No	No	No	1:25	10
10B	2	0	No	91	179	88	Yes	No		1:50	8
11B	[7]	0	No	[4]							
13B	2	0	No	79	167	88	Yes	Yes	No	1:400	15
14B	1	0	No	[3]					No	1:400	10
15B	2	0	No	12	290	278	No	No		1:100	8
16B	2	0	No	29	284	313	No	No	No	1:100	15
19B	1	0	No	101	199	98	Yes	Yes	Yes	1:400	19
20B	2	1	No	2	232	230	[8]	Yes	Yes	1:400	15
21B	1	0	No	85	175	90	Yes	Yes	Yes	1:400	13
22B	2	0	No	[3]					No	1:100	13
24B	2	0	No	[3]					No	1:400	14

[1] Two drops of a heavy suspension of *Br. abortus* were instilled into an eye of each animal listed as exposed.
[2] Exposed but not pregnant.
[3] S=slight reaction.
[4] Not exposed.
[5] This cow aborted in April 1931, 4 months before herd 2 was assembled. No *Br.* organisms were recovered at that time.
[6] *Br. abortus* was isolated from the colostrum of the left rear quarter of the udder at calving on October 30,1932. The serum of the colostrum was positive (1:100) in all quarters. No organisms were isolated at the next calving.
[7] This heifer became a reactor late in November 1931, and was removed from the herd. She calved normally on May 23, 1932. No organisms were recovered at the time of calving.
[8] A premature but living calf was produced.

As will be noted from the data in table 1 (columns 2, 3, and 4)' all calvings previous to the exposure were normal and the pathogen of Bang's disease was not isolated from any individual, except from the colostrum of cow 36 (a ceased reactor) in October 1932, and at the one calving only. Furthermore, although there were slight fluctuations in the agglutinin titer of the sera from the ceased reactors, these were never higher than suspicious reactions.

Shortly after the herd was assembled, the serum of cow 11B reacted with the organism at a dilution of 1:100, and she was immediately removed from the herd and isolated. Although *Brucella abortus* was not recovered following birth at full term of a normal calf, the probability that she was infected cannot be denied. The only animal which, within the writers' knowledge, might have been a carrier of the organism was No. 36, but whether the presumed infection of No. 11B came from this particular cow, or was latent at the time of purchase, cannot be stated definitely.

Four animals (18, 5A, 6A, and 7A) were taken out of the herd, and three (58A, 61A, and 63A) were added about 3 months before the exposure Each of the three replacements had calved shortly before they were placed in herd 2, and came from a herd free of Bang's disease.

There were 34 cows in the herd at the time of the artificial exposure on September 5, 1933. Of these, 13 were ceased reactors, and 21 were normal individuals, except for 23A, as previously described. These were given by way of the eye two drops of a heavy suspension of *Brucella abortus*. Also, a reacting cow which had recently aborted was put in with these animals to allow further exposure by contact. Four of these animals (8, 14B, 22B, and 24B) presumably were not pregnant at the time of exposure. The serum of one of these (No. 8) showed no appreciable increase in agglutinin titer following exposure, while that of the others gave definite evidence of infection; the agglutination titers of their respective sera increased markedly after the exposure, as shown in table 1.

Only one abortion was noted (No. 5) in the group of ceased reactors which were pregnant when exposed. This cow became a definite reactor at approximately 8 weeks after the exposure, and aborted a fetus on the sixty-fifth day after inoculation. *Brucella abortus* was demonstrated in the material examined. A post-mortem examination when this animal was killed, approximately 2 months after the abortion, revealed a severe (pus) infection of both kidneys. Whether this infection was a major contributing factor in the break-down of whatever immunity this cow possessed as a result of the first infection, is not definitely known. About 2 months after the date of the first exposure in 1928 (*11*) the serum of this animal produced a slight agglutination of the organism at a dilution of 1:100, and the organism was not recovered at the time of calving, nor thereafter. It cannot therefore be stated definitely that this animal was not infected at the first exposure, although that may be the explanation for the abortion following the second infection when at least some degree of resistance would have been anticipated as a result of the previous exposure.

One of the ceased reactors (No. 8) presumably did not conceive. Each of the other 11 cows of this group (4, 12, 17, 28, 31, 33, 35, 36, 38, 40, and 44) of ceased reactors produced living calves at full term, and *Brucella abortus* was not recovered from any of them. Only 3 of these 11 cows (28, 36, and 38) showed even suggestive evidence of infection, as indicated by a slight increase in the agglutinating titer of their respective sera for the organism. The sera of the other 8 cows only occasionally, if ever, produced complete agglutination of the organism at a dilution of 1 in 25.

Thus it would appear that although the first exposure (*11*) of these 12 cows was made 5 years before the second, the resistance induced in each by the first infection was sufficient to protect against a subsequent exposure, except for 1 individual (No. 5). Each of these cows had produced either 3 or 4 calves following the calving or abortion subsequent to the initial exposure, and prior to the second.

As stated above, cow 23A was in a herd which, several years previously, had suffered an outbreak of Bang's disease. This herd, however, was free of the disease previous to her transfer to this experiment. The entire lack of any symptons on the part of this

animal (i. e., no increase in agglutinins and no organisms recovered at calving) subsequent to exposure aroused suspicion, and a reexamination of the records of the herd from which she came elicted the above information.

Excluding this individual, among the group of 17 susceptible cows there were 5 which calved normally, 11 which aborted their fetuses at various stages of pregnancy, and one (20B) which produced a living but premature calf. *Brucella abortus* was not recovered at calving from any of the 5 which carried their calves full term; 3 of these (32A, 15B, and 16B) were definite but low reactors at serum dilutions of 1:100, and the sera of the other two (63A and 9B) never produced agglutination at a dilution higher than 1:25.

In the group of susceptible animals, only 1 (2B) of 11 individuals which aborted showed a serum reactivity less than that considered as indicative of a definite reactor. All others were definitely reactors. The organism was not recovered from 3 of these (58A, 2B, and 10B) by the usual guinea pig inoculations following the respective abortions. It is therefore possible that the abortion of 2B was not due to *Brucella abortus*, although it is assumed that the abortions of the other 10 individuals were due to the pathogen.

It is of interest to note that two cows (63A and 16B), which were bred for the final service after the induced exposure, calved normally. Edgington and Donham (*8*) have recently reported, with greater numbers, similar observations, to the effect that cows infected prior to pregnancy are not likely to abort.

At the time of slaughter of these animals, minced tissues from the supramammary lymph gland, minced udder tissue, and washings from the udder of each were injected into guinea pigs in an attempt to determine whether *Brucella abortus* was present. As will be seen in table 1, the organism was not found in all the individuals from which it had previously been recovered at abortion or calving. However, it was found at slaughter only in animals from which it had been recovered at abortion or calving.

HERD 3

Approximately 8 months after the exposure of the cows in herd 2, there were 8 individuals (23A, 32A, 58A, 63A, 9B, 10B, 15B, and 16B) from the previously susceptible group whose sera showed either very low titers or no agglutinins for *Brucella abortus*. The sera of 3 of these (23A, 63A, and 9B) had shown no reactions with complete agglutination of the organisms at a dilution higher than 1:25; the sera of the others had produced agglutination, but at relatively low dilutions. The other animals of the herd were disposed of, the barn and premises were thoroughly cleaned and disinfected, and 28 virgin heifers were added in August 1934 to make herd 3. Cow 16B developed acute mastitis and was removed a few weeks after herd 3 had been assembled.

Table 2 (columns 2 and 3) gives the number of normal pregnancies or abortions for each individual in the herd from August 1934 until the artificial exposure in May 1937. Five individuals (4C, 5C, 17C, 19C, and 21C) were exchanged in April 1936 for five others (19D, 22D, 33D, 38D, and 42D) from another experiment within the station, and six animals (16C, 22C, 26C, 31C, 33C, and 19D) were discarded

for various reasons, none of which was attributable to infection with *Brucella abortus*. Three heifer calves (3H, 4H, and 5H from cows 27C, 63A, and 32A, respectively) which reached sexual maturity prior to the artificial exposure, were bred and then exposed with the others, and data are given concerning their subsequent reactions.

TABLE 2.—*Results obtained with the animals of herd 3 prior to and following their artificial exposure* [1] *to* Brucella abortus

Cow No.	Preexposure period			Postexposure period				*Brucella abortus* recovered—		Agglutination reaction and time observed	
	Number of pregnancies completed		*Brucella abortus* recovered	Days pregnant—		Days from exposure to abortion or calving	Aborted	At calving or abortion	Following slaughter	Highest titer	Weeks following exposure
	Normal	Abortion		When exposed	When aborted or calved						
23A	2	0	No	72	[2]					[3]	
32A	2	0	No	69	280	211	No	No	No	50	2
58A	2	0	No		[2]					50	2
63A	2	0	No		[2]				No	50	2
9B	3	0	No	80	281	201	No	Yes		50	2
10B	2	0	No	80	275	195	No	No		50	2
15B	2	0	No	91	278	187	No	No	No	100	2
1C				[4]							
2C	2	0	No	65	134	69	Yes	Yes	No	400	5
3C	2	0	No	69	181	114	Yes	Yes	No	400	16
4C	1	0	No	[5]							
5C	1	0	No	[5]							
6C	2	0	No	84	188	104	Yes	Yes	Yes	400	5
7C	1	1	No	30	179	149	Yes	Yes	Yes	400	9
8C	2	0	No	64	110	46	Yes	Yes	Yes	400	12
11C	2	0	No	90	183	93	Yes	Yes	Yes	400	5
12C	2	0	No	83	173	90	Yes	Yes	No	200	14
13C	2	0	No	93	191	98	Yes	Yes	Yes	400	3
14C	1	0	No	76	138	62	Yes	Yes	Yes	400	9
16C	2	0	No	[5]							
17C	1	0	No	[5]							
18C	1	1	No	34	240	206	[6]	Yes	Yes	400	11
19C	1	0	No	[5]							
20C	2	0	No	68	281	213	No	Yes	No	[3]	
21C	1	0	No	[5]							
22C	0	0	No	[5]							
24C	2	0	No	81	135	54	Yes	Yes	No	200	4
25C	2	0	No	94	202	108	Yes	Yes		400	10
26C	1	1	No	[5]							
27C	2	0	No	71	281	210	No	Yes		400	16
28C	2	0	No	13	212	199	Yes	Yes	Yes	400	9
29C	2	0	No	79	213	134	Yes	Yes	Yes	400	9
30C	2	0	No	80	202	122	Yes	Yes	Yes	400	17
31C	1	0	No	[5]							
33C	1	0	No	[5]							
19D	1	0	No	[5]							
22D	1	0	No	65	268	203	No	Yes	Yes	200	10
33D	1	0	No	58	187	129	Yes	Yes		400	18
38D	1	0	No	178	251	73	Yes	Yes		200	8
42D	1	0	No	[5]							
3H				71	186	115	Yes	Yes	Yes	400	9
4H				18	270	252	No	No	No	100	8
5H				61	191	130	Yes	Yes	Yes	400	12

[1] Cows 23A, 32A, 58A, 63A, 9B, 10B, 15B, 14C, and 38D were exposed by way of the eye; the others of the herd were given per os 20 cc. of a heavy suspension of *Br. abortus*.
[2] Exposed but not pregnant.
[3] No reaction.
[4] Not exposed; see text.
[5] Not exposed.
[6] Small calf, lived 9 days.

There were 31 different cows which, prior to the induced exposure, had produced either 1 or 2 calves each while in contact with the 7 cows that had recovered from Bang's disease. Within this group and

during this period there were 3 abortions (7C, 18C, and 26C), but these presumably were not due to *Brucella abortus* since the organism was not detected in any of the 3, nor were there demonstrable agglutinins in their sera at a titer above 1:25.

At the time of the artificial exposure in May 1937 there were 31 individuals in the herd, including the 3 heifers mentioned. The 7 ceased reactors and 2 of the susceptible group (14C and 38D) were exposed by instilling 2 drops of a heavy suspension of *Brucella abortus* into the eye. The remaining susceptible animals were given per os 20 cc. of a heavy suspension of the same organism. The adequacy of the infection is attested by the fact that, of the 24 animals of the susceptible group, 19 aborted. There were 4 which produced normal calves at full term, and 1 (42D) presumably was not pregnant. The results of the guinea-pig inoculations with colostrum and uterine exudates at the time of calving or abortion, as given for each animal in table 2, show that the organism was present in each of the 19 susceptible animals that aborted, and also in 3 of the 4 individuals that calved normally.

Four of the seven cows of the ceased reactors produced calves, and these were all carried to full term. *Brucella abortus* was recovered from the uterine fluid of only one of these, cow 9B. The sera from two cows that did not produce calves (58A and 63A) showed a slight rise in agglutination titers very shortly after the exposure.

After slaughter, tissues from 23 of the infected animals were examined for the presence or absence of *Brucella abortus*. The same technique was used as was described for animals of herd 2; i. e., minced tissues from the supramammary lymph gland, from the udder, and washings from the udder were injected into guinea pigs. These tests were made during a period of 21 to 27 months following the exposure. As was found in comparable tests on the individuals of herd 2, not all the animals harbored the organisms which had shed them previously at the respective calvings or abortions. But the organisms were found in the tissues of only those animals from which they had previously been recovered.

An observation of considerable interest was made following the exposure. One cow (No. 1C) calved shortly before the other animals were exposed in May 1937, too late to be treated with them. This cow thereafter was stabled at one end of a row of stanchions which also held infected animals, but was never turned loose with them. During the summer and early fall months, she stood next to an infected cow for an hour or more two or three times a week. As the weather became cooler, all the cows were kept in the barn, except for a few hours a day when the infected animals were let loose in the yard.

No evidence of infection of 1C was noted until December 1937. The serum of this individual had been used weekly as a source of complement for bactericidal tests made on the infected animals, and a reaction out of the ordinary in these tests was noted on December 23, although no appreciable change in the agglutinin titer of the serum for *Brucella abortus* could be detected. Very shortly after this, however (on January 5, 1938), her serum showed a perceptible increase in agglutinins (giving complete agglutination at a dilution of 1:100), and it was learned that several days previous to December 23 this individual had been loose in the barn for several hours, thus

allowing contact with the infected animals. However, it cannot be stated definitely that the exposure was made only at this time. She was bred in February 1938, and produced a normal calf at full term, thus adding another to the examples given above, and to others cited by Edgington and Donham (*8*), that animals infected before pregnancy are likely to produce normal calves.

GENERAL CONSIDERATIONS

The combined results on the ceased reactors in herds 2 and 3 show that, of a total of 16 such individuals which were subjected to a second exposure, 15 produced normal calves at full term and 1 aborted. *Brucella abortus* was isolated at calving from the uterine exudate of only 1 of the 15 animals which produced the normal calves. Huddleson and his coworkers (*12*) were unable to detect the organism in 12 animals which had ceased to react. It appears probable, therefore, that the percentage of ceased reactors which may be found to harbor the organism will be low.

The comparative titers of the sera of the different ceased reactors to *Brucella abortus*, following the first and second exposures, respectively, have been summarized in table 3. The sera of six of these individuals showed an appreciably lower titer following the second exposure than was found during the first infection. There were nine animals, however, whose sera during the second infection showed difference in the height of the titer of only one dilution from that noted in the first infection, probably not a significant difference, and one individual (No. 5) which gave a higher agglutination titer following the second exposure. Most of this latter group of animals had never shown complete agglutination of the organism at a serum dilution higher than 1:100 during either the first or second infection.

TABLE 3.—*A comparison of the agglutination titers for* Brucella abortus *of the sera of animals for the first and second infections,*[1] *respectively, with the pathogen*

Cow No.	Highest titer during infections following—		Cow No.	Highest titer during infections following—	
	First exposure	Second exposure		First exposure	Second exposure
4	[2] S-1:100	S-1:100	36	1:100	1:50
5	S-1:100	1:200	38	1:200	1:100
12	1:200	1:25	40	S-1:200	1:25
17	1:200	S-1:100	44	1:100	S-1:100
28	1:100	1:50	32A	1:100	1:50
31	S-1:100	1:25	9B	1:25	1:50
33	1:200	1:25	10B	1:50	1:50
35	1:200	1:25	15B	1:100	1:100

[1] As a result of changes made in the sensitivity of the antigen over the years in which these tests were carried out, it seems probable that the respective agglutination titers for the sera from the different individuals recorded at the different times of testing are only relatively comparable.
[2] S indicates a slight reaction at the dilution shown.

The results obtained with the ceased reactors in the two different herds indicate that only a relatively small proportion of individuals exposed to a virulent culture of *Brucella abortus* immediately preceding or during pregnancy will be susceptible to a subsequent exposure. Furthermore, the demonstration (*13*) that the serum of the ceased reactors possessed bactericidal antibodies in the absence of agglutinins

beyond the titer of normal cattle adds support to the conclusion that an active immunity is engendered by an infection. Indirectly, then, all such data support the conclusions based upon experiments in vaccination with living cultures by various workers [Buck (*3*), Buck et al. (*4*), Cotton, Buck, and Smith (*5*), Delez (*7*), and Haring (*10*)] that vaccination induces a definite immunization in the treated animals.

SUMMARY

Only 1 of 16 ceased reactors in 2 different herds aborted its fetus when subjected to a second exposure. Following a reexposure, *Brucella abortus* was isolated at parturition from only 1 other ceased reactor. In general, the agglutination titers of the sera of the different ceased reactors were either lower or approximately the same during the second as during the first infection. These results, combined with others on this general subject obtained elsewhere, add to the general belief that an active immunity to *Brucella abortus* is engendered by an infection with virulent organisms. From the experience obtained in these experiments, it appears that the immunity thus engendered is of relatively long duration.

LITERATURE CITED

(1) Beach, B. A., Irwin, M. R., and Ferguson, L. C.
1940. The significance of the "ceased" reactor to Bang's disease. Jour. Agr. Res. 61: 75–80.
(2) Birch, R. R., and Gilman, H. L.
1926. The agglutination test as an aid in handling Bang abortion disease. Cornell Vet. 16: 127–132.
(3) Buck, J. M.
1930. Studies of vaccination during calfhood to prevent bovine infectious abortion. Jour. Agr. Res. 41: 667–689.
(4) Buck, J. M., Cotton, W. E., and Smith, H. E.
1938. Vaccination of calves and yearlings against Bang's disease. U. S. Dept. Agr. Tech. Bul. 658, 7 pp.
(5) Cotton, W. E., Buck, J. M., and Smith, H. E.
1934. Further studies of vaccination during calfhood to prevent Bang's disease. Amer. Vet. Med. Assoc. Jour. 85: 389–397.
(6) Crawford, A. B., and Beach, B. A.
1940. Ineffectiveness of proprietary remedies and other drugs in the control of Bang's disease, with special reference to "3–v tonic" and "Bowman's." Jour. Agr. Res. 60: 565–574.
(7) Delez, A. L.
1937. Results of administering two calfhood injections of a living brucella abortus culture to prevent Bang's disease. Amer. Vet. Med. Assoc. Jour. 90: 61–65.
(8) Edgington, B. H., and Donham, C. R.
1939. Infection and reinfection experiments with Bang's disease. Jour. Agr. Res. 59: 609–618.
(9) Giltner, W.
1929. The present status of infectious abortion control from the laboratory standpoint. Amer. Vet. Med. Assoc. Jour. 74: 880–884.
(10) Haring, C. M.
1938. Vaccination against Bang's disease in an infected dairy herd with United States Bureau of Animal Industry brucella abortus strain 19. Amer. Vet. Med. Assoc. Jour. 92: 52–60, illus.
(11) Hart, E. B., Hadley, F. B., and Humphrey, G. C.
1932. The relation of nutrition to contagious cattle abortion. Wis. Agr. Expt. Sta. Res. Bul. 112, 45 pp.

(12) HUDDLESON, I. F., TORREY, J. P., and CARLSON, E. R.
1928. THE RELATION OF THE AGGLUTINATION TEST TO THE PRESENCE OF BRUCELLA ABORTUS IN THE BODY OF THE BOVINE. Amer. Vet. Med. Assoc. Jour. 72: 889–901, illus.

(13) IRWIN, M. R., and FERGUSON, L. C.
1938. INCREASE IN BACTERICIDINS IN THE SERUM OF CATTLE FOLLOWING RECOVERY FROM INFECTION WITH BRUCELLA ABORTUS. Soc. Expt. Biol. and Med. Proc. 38: 451–452.

U. S. GOVERNMENT PRINTING OFFICE: 1942